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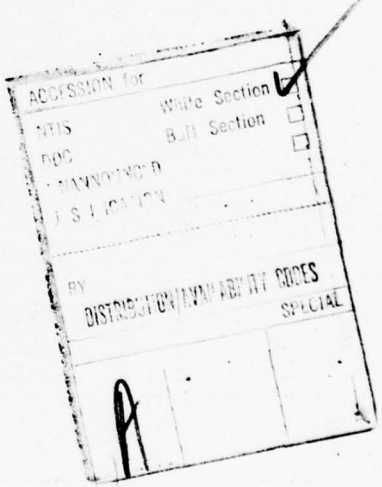
The potential physiological significance of these results includes: possible potentiation of the host's defense mechanisms by Zn^{2+} and its utilization for prolongation of fever to determine its effect on potentially temperature-dependent host defense mechanisms.



In Vitro and In Vivo Actions of Zinc Ion Affecting Cellular Substances Which Influence Host Metabolic Responses to Inflammation

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3 Text Figures

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

ABSTRACT Glycogen-stimulated rabbit peritoneal exudate cells (polymorphonuclear leukocytes, PMN) produce prostaglandins (PG) and substances which induce alterations (mediators) in experimental animals characteristic of host metabolic responses to infection and other acute inflammatory stresses. The effect of Zn^{2+} on mediator production and PG synthesis was examined because: Zn homeostasis is perturbed during infection, Zn is known to regulate some cellular functions, and there appears to be an interrelationship between PG synthesis and mediator production.

Using exudate cells, 2 mM Zn^{2+} caused complete inhibition of in vitro PG synthesis as assessed by conversion of [1-^{14}C]arachidonic acid into PG. This concentration of Zn^{2+} also inhibited production of substances mediating plasma Zn depression, hepatic amino acid "uptake," fever, and neutrophil release from bone marrow. Conversely, Zn^{2+} did not inhibit in vivo metabolic responses to these mediators. Zn-pretreatment of rabbits or simultaneous injection of Zn^{2+} and crude PMN-derived pyrogenic activity resulted in prolongation of fever. It is suggested that this action of Zn^{2+} may be attributed to stabilization of cyclic AMP through inhibition of phosphodiesterase, since analogous experimental results were obtained using theophylline.

The potential physiological significance of these results includes: possible potentiation of the host's defense mechanisms by Zn^{2+} and its utilization for prolongation of fever to determine its effect on potentially temperature-dependent host defense mechanisms.

Zn has been shown to influence many functions of various cell types associated with the inflammatory process. Some of the regulatory functions of Zn observed either in vivo or in vitro include: mitogenic activity for lymphocytes (Chvapil, '76), inhibition of platelet aggregation and serotonin release (Chvapil, '75), prevention of mesenteric mast cell disruption induced by anaphylactic responses to several substances (Hogberg and Uvnas, '60), inhibition of histamine release from mast cells (Kazimierczak and Maslinski, '74), inhibition of macrophage and polymorphonuclear leukocyte (PMN) migration and phagocytic activity (Chvapil, '76), inhibition of granulocyte oxygen consumption and bactericidal activity against Escherichia coli (Chvapil, '77), and intracellular activities too numerous to mention. Of these regulatory functions, the effect of Zn on granulocyte activities is of primary interest to this laboratory because: Zn homeostasis is perturbed in man and experimental animals as a sequel to infection and other acute inflammatory stresses (Beisel, '75), and stimulated phagocytic cells produce substances which induce several metabolic alterations that have been postulated to be part of the host's defense mechanisms against infection (Beisel, '75).

Substances which induce metabolic alterations in experimental animals comparable to those observed in infected men are prepared routinely from glycogen-stimulated rabbit peritoneal PMN. The metabolic alterations induced in rats by these substances include: decreased serum Zn (Kampschmidt and Upchurch, '70) and iron (Kampschmidt and Upchurch, '69) concentrations, increased Zn and iron concentrations within the liver (Pekarek et al., '72b) increased serum copper and ceruloplasmin concentrations (Pekarek et al., '72a), increased release

of neutrophils from bone marrow (Kampschmidt et al., '72), a "flux" of amino acids into the liver (Wannemacher et al., '72b), increased serum α_1- and α_2-acute-phase globulins (Eddington et al., '71), increased RNA synthesis (Wannemacher et al., '75b), and decreased portal vein glucose concentrations accompanied by increased concentrations of glucagon and insulin (George et al., '75). The crude substances derived from stimulated PMN also induce febrile responses which are bioassayed routinely in rabbits (Beeson, '48). Although definitive studies concerning the physiochemical nature of these PMN-derived substances are not available, recent reports suggest that some of the metabolic responses may be induced by lipids (Mapes and Sobocinski, '77b) or essential lipid moieties, some of which are formed as a consequence of prostaglandin (PG) synthesis (Mapes et al., '77).

It, thus, was of interest to determine whether Zn: affects the in vitro production of leukocytic mediators and PG, and might have potential efficacy in control of metabolic responses whose mechanisms of action are known to involve de novo PG synthesis. Results of these studies indicate that Zn ion can inhibit the in vitro formation of PG and those PMN-derived substances which induce fever, enhance neutrophil release from bone marrow, depress plasma Zn, and enhance hepatic levels of nonmetabolizable amino acids. Zn ion, however, has no detectable inhibitory effect on the in vivo action of PMN-derived pyrogenic substance(s) whose mechanism(s) of action involve de novo PG synthesis. Instead, it appears to prolong or augment rabbit febrile responses to pyrogen.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 180-220 gm were purchased from Microbiological Associates or Charles-River Breeding Laboratories, Inc. Male Dunning-Fisher rats weighing 160-200 gm were purchased from the same suppliers. Rats were maintained at 22° C with 12-hour intermittent periods of light and darkness. They had access ad libitum to pelleted rat chow and water. Locally purchased male, New Zealand white rabbits were used for pyrogen assays, while rabbits of either sex were used for production of stimulated PMN.

Preparation of cells

Stimulated rabbit PMN were harvested from glycogen-induced peritoneal exudates and incubated in physiological saline (10^8 PMN/ml) using previously reported procedures (Mapes and Sobocinski, '77a). After 1 hour incubation at 37° C in a shaking water bath, the cellular debris was removed by ultracentrifugation. The supernatant solutions obtained in this manner represent the crude mediator preparations used for subsequent bioassays. All procedures were carried out as aseptically as possible to minimize endotoxin contamination. Individual preparations were tested for endotoxin contamination using pyrogen assays in non-endotoxin refractory rabbits (Greisman and Woodward, '70).

Incubation of cells with Zn ion

Incubations to test the effect of Zn ion on mediator production consisted of 3×10^9 PMN and the desired concentrations of Zn ion, added as $ZnCl_2$ (Mallinckrodt), in 30 ml physiological saline. Control incubations also contained 3×10^9 PMN suspended in 30 ml saline. After 1 hour incubation at 37° C, 1.0 ml of saline or 1.0 ml of $ZnCl_2$ solution of the desired concentration was added to Zn-treated and control incubations, respectively. One portion of PMN also was processed without addition of Zn ion for use as a control to determine whether Zn affected preformed mediator activity.

Separate 10.0-ml portions of the PMN preparation were used to determine the effect of Zn ion on PG synthesis. Subsequent to a 10-minute preincubation with the desired Zn ion concentration, three aliquots of [1-^{14}C]arachidonic acid (0.2 μCi each) were added to the cell preparation at time 0, 15, and 30 minutes. Ten minutes prior to terminating the reaction, the solution was made 1 mM in reduced glutathione (Sigma Chemical Co.). Reactions were terminated after a total of 60 minutes incubation by acidification to pH 3.5 with 1 N HCl and addition of ethyl acetate. Total PG fractions were extracted and eluted from Amberlite columns as described by White and Glassman ('74). Control cells were subjected to identical conditions with the exception that Zn ion was added immediately before termination of the reaction.

Bioassays

Fever responses were measured in non-endotoxin refractory rabbits using previously described techniques for pyrogen assay (Mapes and

Sobocinski, '77a). PMN-derived mediators (typically 1.0 ml of a 1:20 dilution prepared with pyrogen-free saline; dilution selected to preclude temperature elevations of more than 1.5° C) were administered intravenously (iv). Unless indicated otherwise, Zn chloride (1.6 mg/kg) or theophylline (30 mg/kg) was administered intraperitoneally (ip) 1 hour prior to mediators.

Plasma Zn, total blood neutrophils, and hepatic amino acid uptake were measured in samples taken from rats 5 hours after ip administration of a 1.0-ml test sample. Plasma zn concentrations, reported as μg/dl, were determined by atomic absorption spectroscopy as previously described (Pekarek and Beisel, '69). Blood leukocytes were counted in a Coulter counter after a 1:500 dilution of the blood with Isoton (Coulter Diagnostics, Inc.) and lysis of the red blood cells with Hematall LA-Hbg Reagent (Fisher Scientific Co.). Total neutrophils followed from a 100-cell differential count of a smear stained with a modified polychrome methylene blue stain (Hema-Tek Stain Pak). Hepatic amino acid uptake (cpm/50 mg wet weight) was measured by the method of Wannemacher et al. ('72b), with the exception that [1-^{14}C]α-aminoisobutyric acid (AIB, New England Nuclear) was used as the nonmetabolizable amino acid. ^{14}C-AIB levels were measured in liver samples perfused clear of visible blood. Bioassay controls consisted of mediator samples that had been heated at 100° C for 30 minutes prior to administration. Data were statistically analyzed by one-way analysis of variance. The significance of differences between means ($P < 0.01$) was determined using Fischer's protected least significant difference (Snedecor and Cochran, '67).

RESULTS[1,2]

Effect of Zn ion on mediator production: Zn-depressing acitivity

Figure 1 compares the Zn-depressing activity produced in the presence of specific Zn ion concentrations with the activity produced by appropriate control incubations. A decrement in production of this activity was detected with Zn ion concentrations of 0.125 and 0.25 mM; although, these preparations still induced statistically significant Zn depressions when compared to their heat-inactivated bioassay controls. In contrast, the activity produced in the presence of 0.5-2.0 mM Zn ion did not induce Zn depressions statistically different from those produced by administration of their corresponding heat-inactivated controls, thereby indicating 100% inhibition of production. The Zn-depressing activity produced by control cell preparations to which equivalent Zn ion concentrations were added subsequent to the incubation period (fig. 1, expt. controls) were identical with the activity produced by stimulated PMN in the absence of added Zn ion.

The latter observation prompted an investigation of the Zn content of the mediator preparations, since one might assume that normal plasma Zn levels would be altered by administration of preparations with high Zn contents. It was found that a minimum of 85-90% of the Zn ion added to the cell incubations was lost during incubation and processing of the cells (presumably removed with the cellular debris). Time course studies showed that the remaining Zn was absorbed into and cleared from the rats' circulation within 3 hours and, thus, did not alter the plasma Zn levels measured 5 hours after ip injection of test samples.

Production of activities inducing neutrophil release and enhanced hepatic amino acid concentrations

Production of these activities also was inhibited by Zn ion (fig. 2); although inhibition did not appear to be a linear, dose-dependent phenomenon. Production of neutrophil-releasing activity (fig. 2A) was not affected by Zn ion concentrations of 0.5 mM or less, as can be seen by comparing the activities produced in the presence of specific Zn ion concentrations with that produced by cells in the absence of added Zn ion. Production of this activity, however, was inhibited markedly by Zn ion concentrations of 1.0 mM and greater. In addition, a slight increment ($P < 0.05$) in the heat-stability of this activity was noted with those preparations produced in the presence of 0.5-2.0 mM Zn ion. In contrast, neutrophil-releasing activity produced by control cell preparations (equivalent Zn ion concentrations were added subsequent to the incubation period) did not differ statistically from that produced by stimulated PMN without added Zn ion.

A similar pattern of inhibition was observed for production of the activity inducing enhanced hepatic levels of ^{14}C-AIB (fig. 2B). The only difference being that production of this activity was inhibited by Zn ion concentrations in excess of 1.0 mM. A statistically insignificant increment of heat-stability also was noted with preparations that contained 1.5-2.0 mM Zn ion. Again, the activity (and its heat-lability) produced by Zn-treated control cell preparations did not differ from the activity produced in the absence of added Zn ion.

Production of pyrogenic activity

Table 1 summarizes the effect of Zn ion on production of pyrogenic activity by stimulated PMN. Production of pyrogenic activity was inhibited in a dose-dependent manner by Zn ion concentrations ranging from 0.25 to 1.0 mM; whereas, a lower Zn ion concentration (0.125 mM) had no detectable inhibitory effect on its production. Pyrogenic activity produced in the presence of 1.0-2.0 mM Zn ion did not induce rabbit fevers which differed statistically from those induced by administration of an equivalent volume of nonpyrogenic saline, thereby, indicating 100% inhibition of pyrogen production. There was no detectable inhibition of pyrogen production in control cell preparations to which equivalent concentrations of Zn ion were added subsequent to the incubation period. These control preparations induced the same amount of fever as the activity obtained from untreated cells (see Table 1); however, rabbit febrile responses were prolonged by mediators obtained from cells incubated with 1.0 mM or more Zn ion. The latter observation could not be attributed to contaminating endotoxin.

Effect of Zn ion on PG synthesis

Since 2 mM Zn ion was adequate for complete in vitro inhibition of detectable mediator production, the effect of this Zn ion concentration on PG synthesis was assessed. Table 2 summarizes the results of these studies. From the calculated percentage of arachidonic acid incorporated into the total PG fraction in the

presence and absence of Zn ion, it can be seen that 2 mM Zn ion results in 85-90% inhibition of PG synthesis. Zn ion had no effect on the quantity of [1-^{14}C]arachidonic acid recovered in the PG fraction when added to the cellular system after the incubation period. PG synthesis also was evaluated in the presence of lower Zn ion concentrations (0.125-1.0 mM); however, the results of these experiments remain equivocal.

Effect of Zn ion on in vivo pyrogenic activity

It was of interest to determine whether Zn might have some application in control of febrile responses, because of the prolongation of fever observed during bioassays designed to evaluate the effect of Zn ion on production of pyrogenic activity. Therefore, the effect of Zn ion in regulation of fever was studied using crude PMN-derived substances containing pyrogenic activity.

As shown in fig. 3A, PMN-derived substances induced monophasic fevers which reached maximal elevations approximately 45 minutes after iv administration. These fevers returned to basal levels within 70-80 minutes and remained there during the subsequent 2-3-hour period, indicating an absence of contaminating endotoxin in the preparations. When rabbits were treated with Zn ion 1 hour before administration of pyrogenic activity (fig. 3A) the normal fever profiles were altered. In this case, maximal fevers of prolonged duration were obtained 1 hour after administration of mediators and the change in fever (Δ° C) appeared to be about 1.4 times those obtained with equal doses of the same mediator preparation in untreated rabbits. When Zn ion (minimum of 0.04

μg/kg) was administered iv with the mediator preparation (not shown in fig. 3A), fevers of prolonged duration were obtained, but the Δ°C did not differ significantly from that induced by the mediator preparation in the absence of added Zn. Fevers of Zn-mediator treated rabbits remained elevated 4~6 hours before returning to basal levels. Administration of an equal concentration of Zn ion, in the absence of mediators, did not result in a detectable fever alteration during the period of bioassay.

These results suggest several possible in vivo actions of Zn ion including stabilization of cyclic AMP, since Zn is a known inhibitor of phosphodiesterase (Cheung, '67). This possibility was tested directly using theophylline which is also a known inhibitor of phosphodiesterase (Nathanson, '77). Treatment of rabbits with theophylline (fig. 3B), administered ip 1 hour before mediators, resulted in prolonged fevers that were qualitatively similar to those resulting from Zn-mediator treatment of rabbits. Treatment of rabbits with an equivalent concentration of theophylline resulted in no significant alteration of fever during the period of bioassay.

DISCUSSION

The results of this study suggest that Zn ion can inhibit some of the in vitro processes of stimulated rabbit PMN which result in production of PG and those leukocytic mediator activities inducing: fever, plasma Zn depression, release of neutrophils from bone marrow, and enhanced hepatic uptake of amino acids. These effects of Zn ion are consistent with several reports which demonstrate that Zn ion inhibits various granulocyte functions. Some of the functions inhibited by Zn ion include: oxygen consumption (Chvapil et al., '77), phagocytosis (Chvapil, '76) which has been proposed as a trigger for leukocytic mediator production (Beisel, '75), and granulocyte killing of E. coli (Chvapil et al., '77).

Zn ion inhibition of some of these functions depends upon increased intracellular Zn content and, in some cases, the presence of other metal ions in the incubation medium (Chvapil et al., '77). In the present study, however, the action of Zn ion appears to be independent of intracellular Zn ion concentrations. Electron microscopic studies of stimulated PMN, prepared by previously described methods (Mapes and Sobocinski, 77a), showed that the PMN rapidly lost integrity during the incubation period. This loss of cellular integrity does not coincide with termination of either PG or leukocytic mediator production (this laboratory, unpublished data).[3] Likewise, the effect of specific metal ions in combination with Zn is difficult to evaluate in these studies because several metal ions normally are present in mediator preparations. These metal ions include: Cu, 0.6 $\pm$ 0.04; Fe, 46 $\pm$ 10.6; Mg, 0.5 $\pm$ 0.03; Ca, 1.0 $\pm$ 0.1; and Cd, 1.0 (μg/dl $\pm$ SE); and K, 1.1 $\pm$ 0.1 mEq/L. Some of these metal ions alter production of leukocytic mediators

(Mapes and Sobocinski, '77a); however, it remains to be determined whether they act in conjunction with Zn or independently.

The inhibitory effect of Zn ion on PG synthesis is also in agreement with an apparent interrelationship between PG synthesis and leukocytic mediator production (Mapes et al., '77) as well as the effect of other metal ions on PG synthesis (Deby et al., '73). The action of metals on PG synthesis is somewhat dependent upon the PG class of interest and the source of the synthetase system. However, it has been found that heavy metals (Au, Ag and Pt) generally inhibit PG synthesis (Deby et al., '73), while other metal ions (Cu and Fe) augment synthesis of certain PG (Lee and Lands, '72; Zurier, '76). The inhibitory effect of Zn ion (and some other heavy metals) may be attributed to either inhibition of enzymes within the PG synthetase complex or to other potential actions.[4] One possibility is that Zn ion inhibits phospholipase A (Wells, '73), thereby, preventing liberation of substrate (primarily arachidonic acid) for PG synthesis. A second possibility, suggested by [1-^{14}C]arachidonic acid recovery studies, is that Zn ion also enhances incorporation of this fatty acid into membranes where it is not available for PG synthesis. However, the unstable nature of arachidonic acid as well as the numerous "transformations" which it might undergo in the cellular system make it difficult to fully assess this possiblity.

In contrast to its in vitro inhibitory action on mediator production and PG synthesis, Zn ion augmented or prolonged rabbit febrile responses induced by endogenous pyrogen. Since fever induction by pyrogen involves de novo PG synthesis accompanied by enhanced levels of cyclic AMP (Dey et al., '74; Dascombe and

Milton, '75), the potential mechanisms by which Zn ion might prolong fever include: stabilization of endogenous pyrogen, stabilization of other fever inducing substances (cyclic AMP or PG), or initiation of an inflammatory response inducing in vivo pyrogen production.

The latter possibility seems remote since there was no detectable alteration in fever in Zn-treated control rabbits during the period of bioassay. Likewise, the possibility that Zn ion stabilizes PG of the E-type through inhibition of 15-prostaglandin dehydrogenase (PGDH) seems remote since we were not able to demonstrate any inhibitory effect of Zn ion on in vitro PGDH activity in other experiments. Thus, the possibility that endogenous pyrogen or some other fever-producing compound was stabilized, seems the most likely explanation for the observed prolongation of fever by Zn ion.

Although we cannot rule out possible stabilization of endogenous pyrogen, the analogous experimental results obtained by administration of mediator preparations in combination with either Zn ion or theophylline suggest that Zn ion prolongs fever by stabilizing cyclic AMP. This hypothesis is supported further by reports demonstrating that Zn ion (Cheung, '67) and theophylline (Nathanson, '77) are inhibitors of phosphodiesterase, whose inhibition can result in accumulation of cyclic AMP which can cause augmentation or prolongation of some cyclic-AMP mediated reactions (Nathanson, '77).

The in vitro inhibition of some granulocyte functions by Zn ion and the ability of Zn ion to alter in vivo fever responses may be of significance if one considers various host responses to infection.[5]

Plasma Zn levels, for example, normally decrease rather than increase in response to infection or inflammatory stresses (Beisel, '75). This decrease in plasma Zn may also indicate a decreased Zn concentration within granulocytes since they respond rapidly to the Zn concentration of the surrounding medium (Chvapil et al., '76), and the majority of the plasma Zn appears to be redistributed to the liver (Pekarek et al., '72b) where it is incorporated into Zn-binding proteins (Sobocinski et al., '77). Decreased plasma Zn concentrations, thus, may assist phagocytic cell functions which have been postulated to be part of the host's defense mechanisms. Two examples of this possibility are: peak phagocytic activity of cells obtained from burn patients coincides with depressed serum Zn levels (Lennard et al., '74), and infection-induced plasma Zn depressions coincide with some enhanced neutrophil functions (Wannemacher et al., '75b). In vivo Zn depressions also may potentiate either production or activity of some leukocyte-derived mediators; however, Zn-depression is not proposed as a trigger for production of these substances. While plasma Zn depression may precede other metabolic and physiologic (fever) alterations in some infections (Wannemacher et al., '72a), fever is normally the first detectable indicator of illness.

The precise effect(s) of fever on the host's defense mechanism, which includes redistribution of Zn, remains unclear. One reason for this enigma may be that many experimental approaches involve either artificial induction of fevers which exceed the normal range of febrile responses (Bennett and Nicastri, '60) or temperature alterations induced prior to infection (Atwood and Kass,

'64). Prolongation of fever with Zn ion (or perhaps theophylline), thus, may prove to be a useful technique for studying the effect of fever on host defense mechanisms that are potentially temperature dependent (e.g., phagocytosis, leukocyte migration, leukocytic mediator activities).

FOOTNOTES

[1]The leukocytic mediators discussed in this paper classically have been defined as heat-labile substances derived from stimulated PMN (Beisel, '75). Those mediator preparations which do not meet the criterion of heat lability normally are considered to be endotoxin contaminated. During these investigations, however, it was discovered that some mediator preparations can be heat-stable, although bacterial endotoxin contamination could not be demonstrated by either bacterial cultures or pyrogen assays in non-endotoxin refractory rabbits. Zn ion has no detectable effect on either PG synthesis or mediator production with those cell preparations which produce heat-stable mediator activities. Since we currently are unable to explain the differences between heat-stable and heat-labile preparations, we have elected to restrict this presentation to those preparations which meet the classic criterion of heat liability.

[2]Sprague-Dawley rats are used routinely for bioassaying mediator activities. Neutrophil-releasing activity, however, must be confirmed in Dunning-Fisher rats because Sprague-Dawley rats have nonspecific neutrophil responses to mediator preparations containing heparin (Filkins and DiLuzio, '68).

[3]These studies have not been done with intact, stimulated PMN. However, it is worth noting that experimental results obtained with intact cells may differ from ours, since release mechanisms as well as "synthetic" processes may be involved in mediator production.

[4]Possible cytotoxic effects of Zn ion have not been discussed, since we obtained no evidence which indicates that Zn ion is cytotoxic at the concentrations employed in these studies. Similar observations concerning the cytotoxic effect of Zn salts on leukocytes also have been presented by Ward et al. ('75).

[5]On the basis of the experimental results obtained in this study and the available literature, it is reasonable to postulate that Zn ion may have some regulatory function(s) in the host's defense mechanisms. These functions, however, may not be as simple as the reader is led to believe, since the effects of Zn ion are extremely complex. Thus, one must also consider possible differences in the effect of Zn ion on resting, partially-stimulated, and fully-stimulated granulocytes; the magnitude of Zn alterations which occur during infection; and the Zn ion concentration range over which a specific effect may be observed.

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TABLE 1

Effect of zinc ion on production of pyrogenic activity

mM Zn^{2+} Added	Δ°C (mean ± SD)	
	Zinc-treated	Control
None	0.9 ± 0.2	-
0.125	0.9 ± 0.2	1.0 ± 0.3
0.25	0.6 ± 0.3	0.9 ± 0.2
0.50	0.4 ± 0.2	0.9 ± 0.3
1.0	0.2 ± 0.2[1]	1.0 ± 0.2
1.5	0.2 ± 0.2[1]	1.0 ± 0.2
2.0	0.2 ± 0.3[1]	0.9 ± 0.2

Rabbit febrile responses to pyrogenic activity obtained from stimulated PMN in the presence (Zn-treated) and absence (control, equivalent Zn ion concentrations were added at the termination of incubation) of various Zn ion concentrations. Individual preparations were tested in 4 rabbits, 45 minutes after iv injection of a 50-µl test sample diluted to 1.0 ml with pyrogen-free saline.

[1]Statistically significant ($P < 0.01$) fevers could not be obtained with increased doses (200-500 µl) of pyrogenic material.

TABLE 2

Effect of zinc ion on utilization of [1-^{14}C]arachidonic acid for prostaglandin synthesis

Preparation	% of Control		% Inhibition
	2 mM Zn^{2+}	Control	
1	0.6	5.3	89
2	0.7	4.4	85
3	0.7	6.4	90

PG synthesis by stimulated peritoneal PMN is summarized for three representative preparations. Inhibition of PG synthesis was determined by comparing the percent [1-^{14}C]arachidonic acid utilized for PG synthesis in the presence of zinc ion with that utilized by control cells to which equivalent zinc ion concentrations were added subsequent to the incubation.

LEGENDS TO FIGURES

Fig. 1. Zn-depressing activity produced in the presence of specific Zn ion concentrations is compared to the activity produced by experimental controls to which equivalent Zn ion concentrations were added subsequent to the 1-hour incubation. Bioassay controls were obtained by heating individual samples at 100°C for 30 minutes prior to administration. Each point represents the mean (± SE) plasma Zn concentration of 10 rats 5 hours after ip injection of a 1.0-ml test sample; shaded areas represent the mean (± SE) plasma Zn concentration of all control animals.

Fig. 2. *Stimulated PMN production of activities* inducing neutrophil release (A) and enhanced hepatic amino acid uptake (B). Activities produced in the presence of specific Zn ion concentrations (open bars) are compared with their corresponding, heat-inactivated bioassay controls (hatched bars). The activity and heat-lability of substances produced by control preparations to which equivalent Zn ion concentrations were added subsequent to the incubation period (data not shown) did not differ statistically from those of the substances produced in the absence of Zn ion. Each bar represents the bioassay mean ± SE obtained from 10 rats 5 hours after ip injection of a 1.0-ml test sample.

Fig. 3. Effect of Zn ion given as $ZnCl_2$ (A) or theophylline (B) on rabbit febrile responses to PMN-derived pyrogenic activity. Fever induced by a typical mediator preparation (◈) is compared with fevers obtained after treatment with, Zn or theophylline and mediator (○), and Zn or theophylline in the absence of mediators (▲). Zn (1.6 mg/kg) or theophylline (30 mg/kg) was administered ip 1 hour prior to iv administration of 50 µl mediator preparation diluted to 1.0 ml with pyrogen-free saline. Hour 0 represents the time of mediator administration. Each point represents the mean response of 4 rabbits.

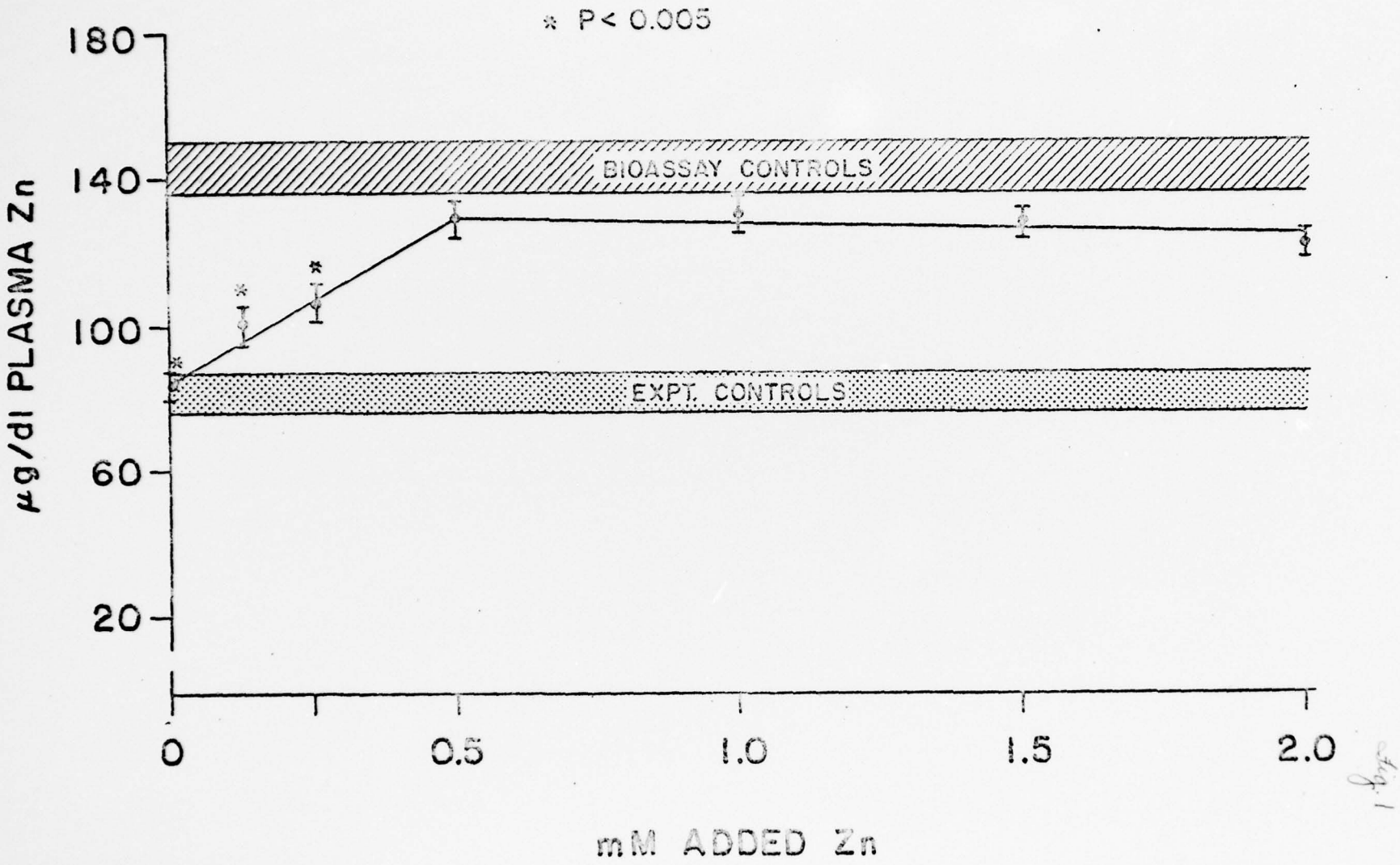

* P< 0.005
180
140
100
60
20
BIOASSAY CONTROLS
EXPT. CONTROLS
μg/dl PLASMA Zn
0
0.5
1.0
1.5
2.0
mM ADDED Zn

Fig. 2

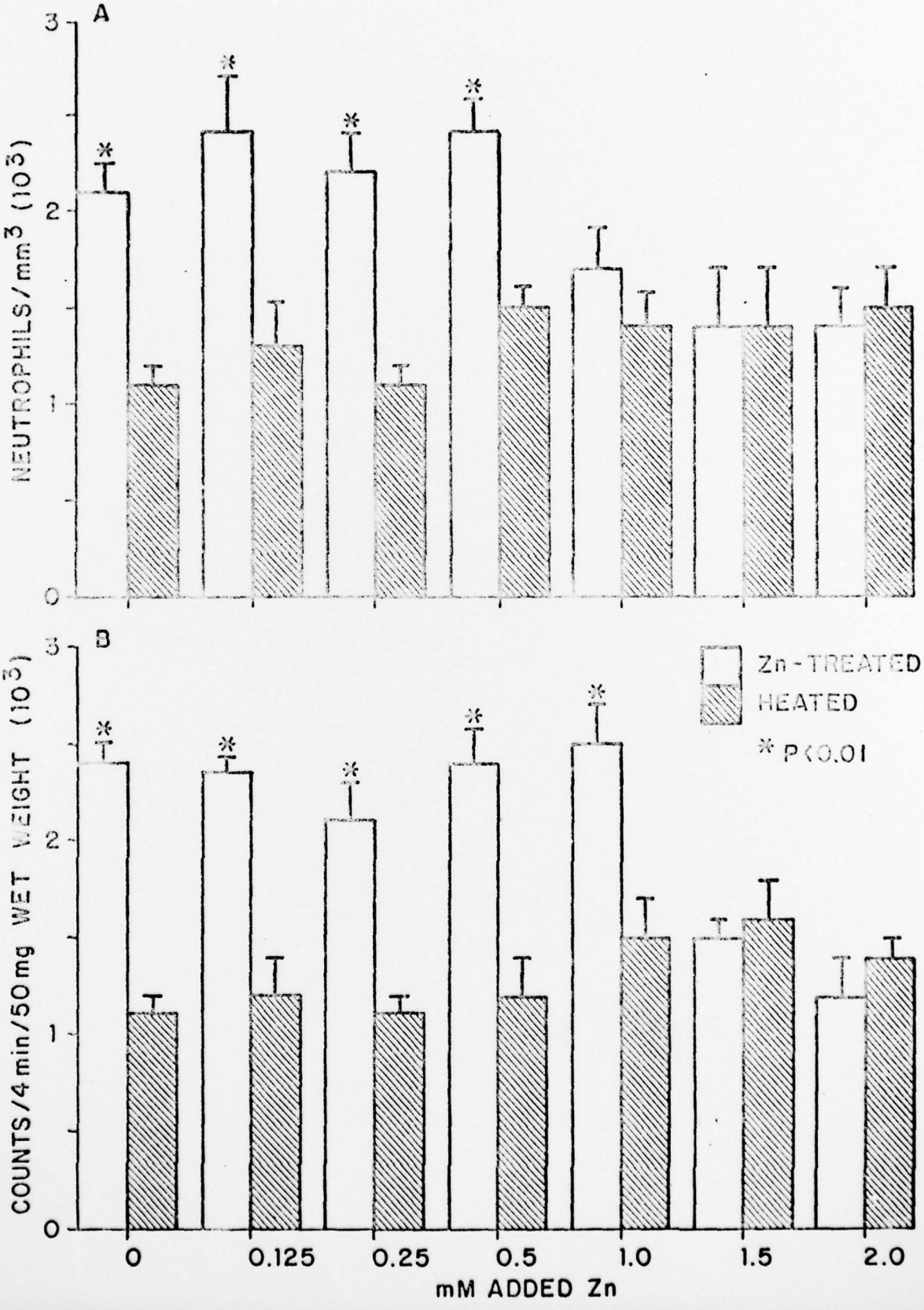
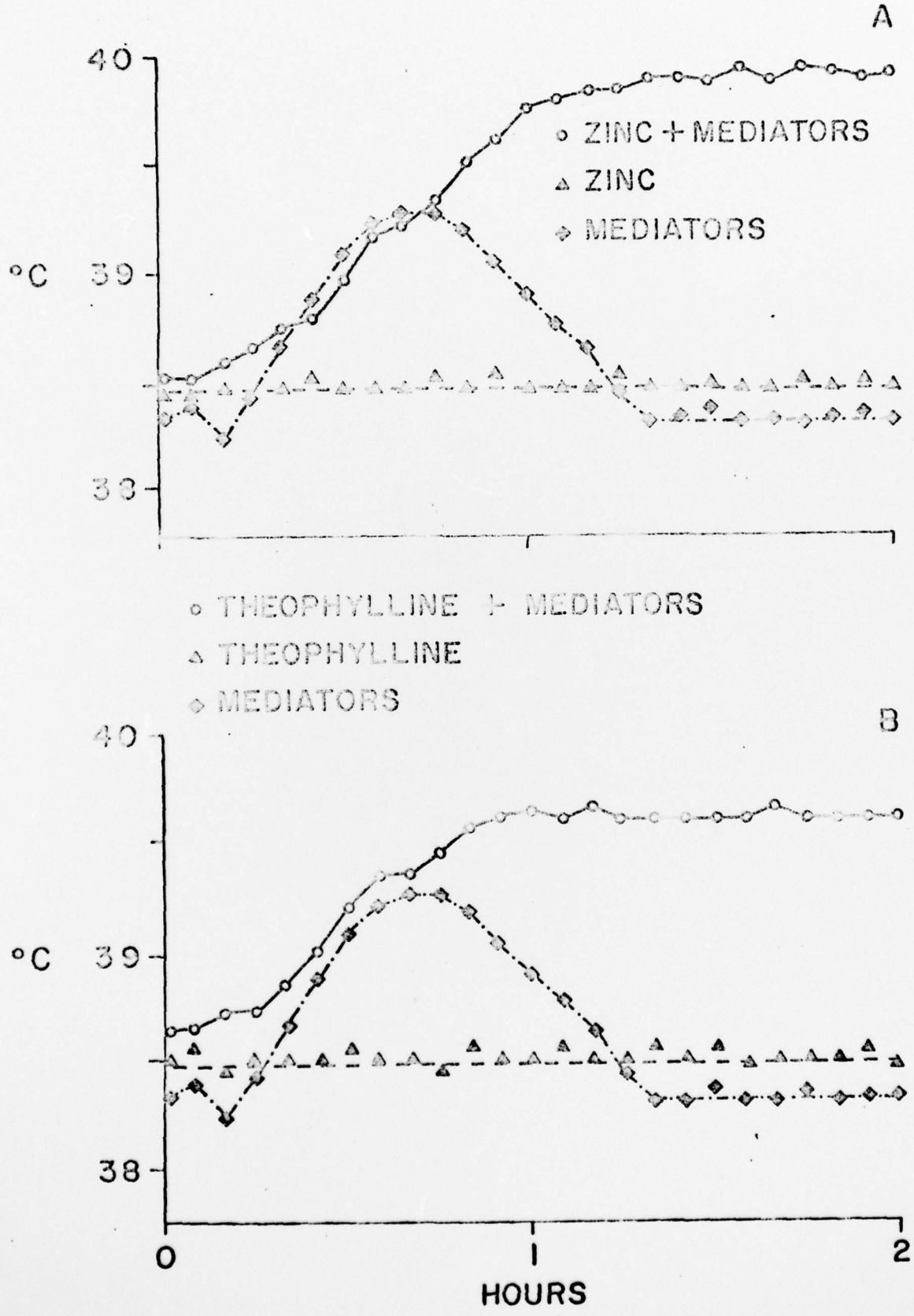

A
NEUTROPHILS/mm3 (10³)
3
2
1
0
B
COUNTS/4 min/50 mg WET WEIGHT (10³)
Zn-TREATED
HEATED
* P<0.01
0
0.125
0.25
0.5
1.0
1.5
2.0
mM ADDED Zn

Fig. 3


A
40
°C 39
38
ZINC + MEDIATORS
ZINC
MEDIATORS
THEOPHYLLINE + MEDIATORS
THEOPHYLLINE
MEDIATORS
B
40
°C 39
38
0
1
2
HOURS